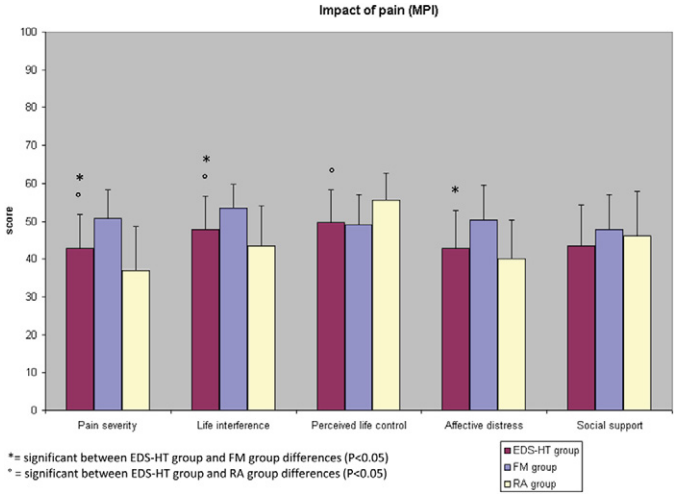

Figure 1

and pain-related suffering, significantly more interference of pain with daily activities, and significantly lower control over pain and life events compared to the RA group. However, in comparison with the FM group, the EDS-HT group showed significantly lower levels of pain severity and life interference, and was less bad-tempered, over-sensitive and anxious ('affective distress'). Social support of significant others due to pain was similar between the 3 groups.

The results in Figure 2 showed clinically relevant health-related dysfunction in all groups. Especially, a significantly poorer physical, psychosocial and overall health function was found in the EDS-HT group compared with the RA group. In contrast, in comparison with the FM group, the EDS group reported a similar physical and overall health status, but a better psychosocial health.

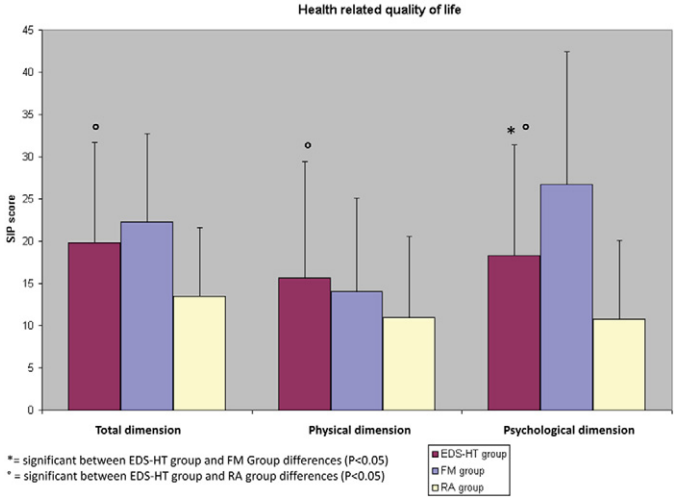

Figure 2

Conclusions: This study demonstrates that patients with EDS-HT experience a broad psychosocial impact of chronic pain on daily life, similar or even worse compared to patients with FM or RA. At the same time, this study shows that EDS-HT has a considerable impact on physical and psychosocial health in most areas of HRQOL. Our findings emphasize that EDS-HT, like other chronic widespread musculoskeletal pain disorders, is associated with a consistent burden of disease. Therefore, treatment of pain should be a prominent aspect of symptomatic management of EDS-HT.

Proteomics & Metabolomics

501

PROTEOMIC PROFILING OF CARTILAGE EXTRACELLULAR MATRIX MATURATION

R. Wilson[1], A. Diseberg[2], S. Zivkovic[1], L. Gordon[1], L. Tatarczuch[3], E. Mackie[3], J. Gorman[2], J. Bateman[1]
[1]MCRI, Melbourne, Australia; [2]QIMR, Brisbane, Australia; [3]Melbourne Univ., Melbourne, Australia

Purpose: In recent years there has been a rapid expansion in the use of proteomics to discover biomarkers and new molecular mechanisms involved in joint disease. In addition, proteomic discovery of novel cartilage components and interactions that have evaded detection by more targeted biochemical approaches will facilitate analysis of biomaterials developed for cartilage repair. To gain a more complete understanding of cartilage extracellular matrix (ECM) maturation, we analyzed changes in protein expression and solubility in a novel mouse neo-cartilage system.
Methods: Primary mouse chondrocytes were maintained in scaffold-free, high density cultures for up to 6 weeks and transmission electron microscopy was used to verify production of a cartilaginous ECM. Sequential extracts of juvenile cartilage (P3) and 3-week neo-cartilage, prepared using 1M NaCl followed by 4M GuHCl, were initially analysed by SDS-PAGE. Label-free quantitative mass spectrometry (LTQ-Orbitrap), statistical and bioinformatic analysis was then used to filter out three significant protein groups: proteins enriched according to extraction condition ("extraction profiling"), proteins differentially abundant between juvenile cartilage and neo-cartilage, and proteins with different solubility properties between the two tissues. Key proteins were further investigated in neo-cartilage and 3-week mouse femoral head cartilage by immunohistochemistry.
Results: Ultrastructural analysis revealed clearly defined pericellular and territorial matrix zones, dense proteoglycan/collagen networks and intricate chondrocyte-matrix contacts. SDS-PAGE indicated that in juvenile cartilage, more proteins were readily soluble, whereas in the neo-cartilage more proteins were extracted under denaturing conditions. LTQ MS/MS identified a total of 819 proteins at high confidence (2 or more unique peptides), of which 620 and 706 proteins were detected in juvenile cartilage and neo-cartilage, respectively. Proteins significantly enriched in neo-cartilage ($n=78$, $p<0.05$ using Student's t-test) included proteins previously not reported or with unknown function in cartilage (EDIL3, integrin-binding protein DEL1; CCD80, coiled-coil domain-containing protein 80; EMIL1, elastin microfibril interface-located protein 1 and PEDF, pigment epithelium-derived factor). The cohort of proteins with the greatest differential in extractability between the two sample types included many pericellular and extracellular matrix components, including collagen VI, nidogen-2, perlecan, matrilin-3 and COMP. One of the guanidine extract specific proteins in the mouse neo-cartilage was the serine protease inhibitor, protease nexin-1. We confirmed PN-1 as a novel component of developing articular cartilage in vitro and in vivo by immunohistochemistry.
Conclusions: The cartilage "extraction profiles" are, to our knowledge, the most detailed solubility-based comparative analysis of a tissue proteome. The partitioning of readily soluble proteins from more tightly-integrated components applied to cellular components of large protein complexes (eg tubulins, ribosomal and proteasomal subunits) as well as ECM proteins and proteoglycans. This fractionation approach therefore facilitates deeper mining of the proteome while maintaining important biochemical information related to the proteins identified. Using these differences in protein solubility we generated a comprehensive profile of mouse neo-cartilage and identified novel components involved in maturation of the cartilage ECM.

502

STUDY OF THE EFFECT OF CHONDROITIN SULFATE ON CARTILAGE EXTRACELLULAR MATRIX METABOLISM BY CHONDROCYTE SECRETOME ANALYSIS: USEFULNESS OF A QUANTITATIVE PROTEOMIC APPROACH

V. Calamia[1], B. Rocha[1], P. Fernandez-Puente[1], J. Mateos[1], E. Montell[2], J. Verges[2], C. Ruiz-Romero[1], F.J. Blanco[1]
[1]Osteoarticular and Aging Res. Lab. Proteomic Unit-Associated Node to ProteoRed. INIBIC-Complejo Hosp. Univ. A Coruña, A Coruña, Spain; [2]Med. Dept. Bioiberica Pharma., Barcelona, Spain

Purpose: To study the effect of chondroitin sulfate (CS) on the profile of